Platelet and endothelial function: Polycystic ovary syndrome and the renin-angiotensin system

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Table of contents

CHAPTER 5

Differential roles of angiotensin-(1-7) and angiotensin II on platelet function 157

CHAPTER 6

List of figures

List of tables

Thesis summary

Background

The phenomenon of platelet hyperaggregability and decreased platelet responsiveness to nitric oxide (also termed as nitric oxide resistance), documented in several cardiovascular disease states, is associated with adverse cardiovascular outcomes.

The series of experiments described in this thesis address primarily some aspects of the pathophysiology, epidemiology and therapy of the phenomenon of end-organ resistance to nitric oxide (NO) in two important conditions, that are closely associated with cardiovascular risk factors and disease states:-

- Polycystic ovary syndrome, which is closely linked with the metabolic syndrome and premature subclinical atherosclerosis.
- The renin-angiotensin system, which is recognized as a significant mediator in the pathophysiology of a number of cardiovascular disease states.

The first study examined the epidemiology/pathophysiology of putative platelet/endothelial dysfunction in young individuals with PCOS. The subsequent studies focused on the potential impact of the renin-angiotensin system on platelet and endothelial function. This mechanistic review is set in the context of a number of recent major clinical studies which have demonstrated surprising efficacy of certain angiotensin-converting enzyme (ACE) inhibitors (ramipril and perindopril) in the prevention of thrombotic processes. Thus we tested the hypothesis whether ACE inhibitor ramipril sensitizes platelets to NO (as a potential mechanism for improved cardiovascular outcomes) in a high risk patient cohort. In addition, particular attention will be given to the emerging role of the heptapeptide Angiotensin-(1-7), a possible physiological antagonist to Angiotensin II in the vasculature and the limitation of the current literature concerning potential effects of the renin-angiotensin system on thrombotic mechanisms.

Study examining platelet and endothelial function in women with polycystic ovary syndrome

Background

Polycystic ovary syndrome (PCOS) is frequently associated with obesity and the metabolic syndrome, which may imply incremental cardiovascular risk. The aim of this study was to determine whether PCOS is associated with platelet and/or vascular endothelial dysfunction, and whether this dysfunction is influenced by the presence of obesity and insulin resistance.

Methods and results

In this pilot study, women with PCOS (n=24; mean age 32 ±1) were evaluated in lean (L-PCOS; n=12) and obese (O-PCOS; n=12) subgroups, and compared with age-matched lean normal women (n=12). Platelet aggregation, its inhibition by nitric oxide (NO) donor sodium nitroprusside (SNP) and vascular endothelial function were assessed. Plasma concentrations of malondialdehyde (MDA), N^G, *NG*-dimethyl-L-arginine (ADMA) and high-sensitivity C-reactive protein (hs-CRP) were measured as markers of oxidative stress, endothelial dysfunction and inflammation respectively. Only one subject with PCOS met the Adult Treatment Panel III diagnostic criteria for metabolic syndrome. Platelet aggregation to adenosine diphosphate (ADP) 1 μM, 2.5 μM and 5.0 μM was 0.4±0.2 ohms, 5.4±0.8 ohms and 6.8±0.8 ohms respectively in the normal group. Comparatively, aggregation was significantly greater in both PCOS subgroups ($p<0.001$ for aggregation at all three concentrations of ADP in both L-PCOS and O-PCOS subgroups). Responses to NO inhibition of aggregation to ADP (2.5 μmol/L) were 40.0±7.5%, 38.4±6.4% and 72.4±6.0% in L-PCOS, O-PCOS and normal groups respectively ($p<0.01$ for both: L-PCOS vs normal and O-PCOS vs normal). Similar reduction in responses was seen at ADP 5 μmol/L in both PCOS subgroups. Endothelium-dependent vascular responses to salbutamol were significantly reduced in both PCOS subgroups compared to the normal cohort [peak responses (measured by peak change in augmentation index) was 1.2±2.1%, 1.9±1.6% and -5.2±1.7% in L-PCOS, O-PCOS and normal groups respectively, $p<0.05$ for both comparisons). Plasma concentrations of MDA were increased in PCOS subjects, independent of subgroup (0.25±0.02 μmol/L, 0.25±0.02 μmol/L and

0.17±0.02 μmol/L in L-PCOS, O-PCOS and normal groups respectively, $p<0.05$ for both comparisons). ADMA ($p<0.01$) and hs-CRP ($p<0.02$) were raised in the PCOS group, primarily due to elevation in the O-PCOS group.

Conclusions and future implications

PCOS subjects, independent of obesity and insulin resistance, exhibit substantial platelet and vascular endothelial abnormalities, as well as evidence of incremental oxidative stress compared to age-matched healthy normal women. These anomalies may represent a significant basis for cardiovascular risk in women with PCOS, independent of metabolic syndrome. Detailed evaluation of the epidemiological and therapeutic implications of these anomalies is therefore of considerable priority. The future directions following these results will be as follows:

- To assess the evolution of these anomalies with advancing age.
- To evaluate the impact of treatment with oral contraceptives and insulin sensitizers, agents frequently used in the treatment of this patient group.
- To elucidate if these anomalies are accentuated with the presence of the metabolic syndrome

The subsequent studies evaluate the following hypothesis:

- Whether ACE inhibitors sensitize platelets to NO (as a potential mechanism for improved cardiovascular outcomes) in a high risk patient cohort**.**
- Whether Ang-(1-7) is a possible modulator of tissue responsiveness to NO, and thus of the phenomenon of NO resistance at the platelet level
- Whether the effect of Ang-(1-7) on platelet NO responsiveness is mediated by an Ang-(1-7) receptor and is platelet-mediated. In addition, the relationship between Ang-(1-7) and oxidative stress is explored.
- Whether the effect of Ang-(1-7) on platelet NO responsiveness is influenced by ACE inhibitor therapy.

Study examining the effects of ACE inhibitors on platelet aggregation and platelet responsiveness to the anti-aggregatory action of Nitric Oxide Donor Sodium Nitroprusside in a high risk patient cohort.

Background

ACEI have previously been shown to have beneficial effects in preventing ischemic coronary events; however, the mechanisms underlying these effects remain uncertain. We tested the hypotheses that (a) ramipril sensitizes platelets to NO in a high risk (HOPE study-type) patient cohort, and (b) that these putative effects of ramipril are more pronounced in patients with platelet NO resistance.

Methods and results

In a double-blind study, 119 patients who either had known ischemic heart disease or diabetes plus additional coronary risk factor(s) were randomized to receive ramipril (n=60; 10mg/day) or placebo (n=59). Whole blood platelet aggregation and its inhibition by the NO donor sodium nitroprusside (SNP) were assessed at baseline and 3 months after randomization. Responses to SNP were analyzed relative to ADP response during the study. Platelet NO resistance was defined as <35 % inhibition of aggregation by SNP. Plasma levels of asymmetric dimethyl arginine (ADMA) were measured as an index of endothelial dysfunction, while augmentation index [AIx] was assessed as a manifestation of arterial wave reflection. Mean patient age was 67±8 (SD) years. Ramipril therapy, while not affecting platelet aggregation, significantly increased SNP response relative to extent of aggregation in the entire cohort ($p<0.001$, ANCOVA). However, increases in unadjusted response to SNP occurred entirely in the NO-resistant patient subgroup (n=49; $\Delta 33.1 \pm 6.2$ vs $\Delta 13.5 \pm 5.1$ % increase for ramipril and placebo groups respectively, $p=0.02$). Ramipril also reduced AIx ($p=0.02$) and ADMA levels ($p=0.05$).

Conclusions

In a HOPE study-type patient population, ramipril sensitizes platelets to NO. This effect largely reflects amelioration of NO resistance in patients with impaired platelet NO response. Reversal of platelet NO resistance may contribute to the beneficial effects of ramipril in patients at high risk of ischemic events.

Study examining effects of Angiotensin-(1-7) and Angiotensin II on platelet aggregation and platelet responsiveness to the anti-aggregatory action of Nitric Oxide Donor Sodium Nitroprusside

Background

Patients with ischemic heart disease have platelets that are resistant to the anti-aggregatory effects of nitric oxide (NO) donors. This resistance is largely a result of accelerated NO clearance by superoxide radical (O_2^-). While Ang II has been shown to augment superoxide formation, recent studies have demonstrated that Ang-(1-7) has potentially opposite actions to those of Ang II in the vasculature. The current study compares the effects of Ang-(1-7) and Ang II on platelet aggregation and platelet responsiveness to the NO donor, SNP.

Methods and results

In whole blood samples obtained from normal subjects (n=17) and patients with acute coronary syndromes (ACS, n=17), platelet aggregation was induced by the thromboxane A_2 mimetic, U46619 (1-5 μmol/L), and ADP (2.5-5 μmol/L). SNP (10 μmol/L) was added 1 minute prior to the addition of agonist and its inhibitory effect on platelet aggregation was quantitated in the presence and absence of Ang-(1-7) (1 nmol/L-1 μmol/L). The effects of Ang II (1 nmol/L-1 μmol/L) were studied in a similar manner in 8 normal subjects. Ang-(1-7) did not significantly affect U46619- or ADP-induced platelet aggregation, but at concentrations of 10-100 nmol/L, potentiated the anti-aggregatory effects of SNP. With U46619 as an agonist, the maximum absolute potentiating effects were 25±4% and 23±3% for normal subjects and patients respectively ($p<0.001$ for both). At higher concentrations (1 μmol/L), this effect was absent. Ang II (10-100 nmol/L) increased U46619-induced platelet aggregation ($p<0.01$), irrespective of presence or absence of SNP.

Conclusions

Ang-(1-7) potentiates the effects of NO in inhibiting platelet aggregation, while Ang II potentiates the proaggregatory effects of thromboxane A_2 mimetic. Thus, these peptides exert biologically "opposing" effects in an indirect manner. It remains uncertain whether these effects, which occurred in vitro at supraphysiological concentrations of both angiotensin peptides, are representative of physiological antagonism.

Study examining the potential role of the Ang-(1-7) receptor in mediating the effects of Ang-(1-7) on platelet NO responsiveness and the relationship between Ang-(1-7) and oxidative stress

Background

Ang-(1-7) potentiates the effects of NO in inhibiting platelet aggregation. The mechanism(s) underlying this observation is unclear. This study is aimed at investigating the putative role of a specific Ang-(1-7) receptor in mediating this effect. In addition, potential relationships between Ang-(1-7) and platelets as well as oxidative stress are explored.

Methods and results

Platelet aggregation studies involving the use of Ang-(1-7) antagonist (Study 5.2A) and oxidative stress (Study 5.2C) were performed in whole blood. Platelet-rich plasma was utilized to assess whether the effect of Ang-(1-7) platelet NO responsiveness is mediated specifically by platelets (Study 5.2B). Ang-(1-7) exerted a concentration-dependent biphasic effect on platelet responses to the inhibitory actions of NO, with a potentiation of NO response at low concentrations (10nmol/L-100nmol/L) ($p<0.01$) and abolition of effect at higher concentrations (1 μmol/L). While pre-treatment with Ang-(1-7) antagonist, D-ala^7-Ang-(1-7) (1 μmol/L) alone did not exert any significant effects on platelet aggregation induced by ADP in the presence and absence of SNP, it completely inhibited the potentiation of Ang-(1-7) on NO response at low concentrations ($p<0.01$). In platelet-rich plasma, Ang-(1-7) did not alter ADP-induced aggregation in the absence or presence of SNP. As regards Study 5.2C, Ang-(1-7) did not significantly alter the intensity of aggregation-induced lucigenin-derived chemiluminescence (LDCL) by ADP (2.5 μmol/L) in whole blood. However, Ang-(1-7) at low concentrations (1nmol/L) significantly ($p=0.024$) potentiated NO inhibition of aggregation-induced LDCL. At higher concentrations (1 μmol/L), there was a non-significant trend towards an opposite effect of Ang-(1-7) on NO inhibition of aggregation-induced LDCL.

Conclusions

Firstly, Ang-(1-7) at low concentrations mediates the potentiation of the anti-aggregatory effects of NO via a specific Ang-(1-7) receptor. Secondly, Ang-(1-7) potentiation of platelet NO responsiveness is not directly mediated by platelets. Thirdly, Ang-(1-7), at low concentrations, significantly attenuates

aggregation-induced superoxide production in whole blood. This study provides the first evidence for the existence of a specific Ang-(1-7) receptor in human whole blood as well as evidence for a relationship between Ang-(1-7) and oxidative stress.

Study examining whether the effects of exogenous Ang-(1-7) on platelet NO responsiveness are affected by the presence of ACE inhibitor therapy

Background

While studies have shown that exogenous Ang-(1-7) has effects on both vascular as well as platelet function, there is little evidence of its benefit in the presence of chronic ACE inhibition therapy in humans. The objective of this study was to evaluate whether supraphysiological concentrations of Ang-(1-7) exert a differential effect on platelet function in the presence of chronic ACE inhibition.

Methods and results

A subset of patients (n=44) who are at high cardiovascular risk (enrolled in the study reported in Chapter 4) were randomized to ramipril (10 mg) (n=22) and placebo (n=22). Platelet aggregation studies were performed in whole blood samples using ADP (2.5 μmol/L) as an agonist. SNP (10 μmol/L) was added 1 minute prior to the addition of agonist and its inhibitory effect was quantitated in the presence and absence of Ang-(1-7) (1 nmol/L-1 μmol/L). Twelve weeks of ramipril therapy did not significantly alter ADP-induced aggregation or platelet responsiveness to NO in this patient population. Ramipril therapy significantly increased endogenous Ang-(1-7) levels and decreased Ang II/ Ang I ratio. Following 12 weeks of therapy compared to baseline, there was no significant change in the effect of Ang-(1-7) on platelet NO responsiveness within each group (p=0.15) and between each group (p=0.60) by 2-way ANOVA.

Conclusions

This study has found no evidence that chronic treatment with the ACE inhibitor ramipril affects the interaction between Ang-(1-7) and platelet responsiveness to NO.

The future directions following these results will be as follows:-

- To examine the physiological significance of Ang-(1-7) on platelet function possibly by administration of a specific Ang-(1-7) antagonist in vivo.
- To explore strategies that could result in incremental concentrations of Ang-(1-7).
- To study further the physiological mechanisms/significance of platelet-leukocyte interactions and Ang-(1-7).

Glossary of abbreviations

Abbreviation	Definition
AA	Arachidonic acid
ACS	Acute coronary syndrome
ADMA	Asymmetric N^G,N^G-dimethyl-L-arginine
ADP	Adenosine diphosphate
AIx	Augmentation index
ANCOVA	Analysis of covariance
Ang	Angiotensin
ANOVA	One-way analysis of variance
ATP III	National Cholesterol Education Program's Adult Treatment Panel III
AUC	Area under curve
BK	Bradykinin
BMI	Body mass index
C	Celsius
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CVD	Cardiovascular disease
DAG	Diacylglycerol
DDAH	dimethylarginine dimethylaminohydrolase
EDTA	Ethylenediamine tetraacetic acid
eNOS	Endothelial nitric oxide synthase
FAD	flavin adenine dinucleotide
FBF	Forearm blood flow
FMD	Flow-mediated vasodilatation
FMN	flavin mononucleotide
Gamma	□
GP	Glycoprotein
H_2O_2	Hydrogen peroxide
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model of insulin resistance
hs-CRP	high-sensitivity C-reactive protein
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IP_3	Inositol 1,4,5-trisphosphate
ISDN	Isosorbide dinitrate
LDL	Low-density lipoprotein

L-NMMA	N^G-monomethyl-L-arginine
L-PCOS	Lean-PCOS
MDA	malondialdehyde
NADPH	nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO^-	Nitroxyl
NO^+	Nitrosonium
NOS	Nitric oxide synthase
GTN	Nitroglycerin
O-PCOS	Obese-PCOS
PAI-1	plasminogen activator-1
PCOS	Polycystic ovary syndrome
PG	Prostaglandin
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKC	Protein kinase C
PLA_2	Phosphalipase A_2
PLC	Phospholipase C
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
PWA	Pulse wave analysis
QUICKI	Quantitative insulin sensitivity check index
ROS	Reactive oxygen species
SAP	Stable angina pectoris
SDMA	N^G,$N^{G'}$-dimethyl-L-arginine
SEM	Standard error of mean
sGC	Soluble guanylate cyclase
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
Superoxide	O_2^-
TBA	Thiobarbiturate acid
TNFα	Tumour necrosis factor α
TxA_2	Thromboxane A_2
TxR	Thromboxane A_2 receptor
VASP	Vasodilator-stimulated phosphoprotein
VSMCs	Vascular smooth muscle cells
vWf	Von Willebrand factor

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the university library, being available for loan and photocopying.

Sharmalar Rajendran
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Publications, presentations and awards

Peer reviewed articles relating to this thesis

Rajendran S, Chirkov YY, Campbell DJ, Horowitz JD. Angiotensin-(1-7) Enhances Anti-Aggregatory Effects of the Nitric Oxide Donor Sodium Nitroprusside. J Cardiovasc Pharmacol 2005; 46: 459-463

Rajendran S, Chirkov YY and Horowitz JD. Potentiation of platelet responsiveness to nitric oxide by angiotensin-(1-7) is associated with suppression of superoxide release. Platelets. 2007 Mar;18(2):158-64.

Accepted presentations at international meetings

S Rajendran, SR Willoughby, EA Kelly, T Heresztyn, R Norman and JD Horowitz. Severe platelet and endothelial dysfunction in women with polycystic ovary syndrome. Accepted for presentation at AHA scientific sessions 2005. (Nominated for Samuel A. Levine Young Investigator Award)

S Rajendran, YY Chirkov, DJ Campbell and JD Horowitz. Differential effects of Angiotensin II and Angiotensin-(1-7) on platelet aggregation. Accepted for presentation in Cardiac Society, CSANZ 2005

Yuliy Chirkov, Sharmalar Rajendran, Aaron Sverdlov, John D Horowitz. Potentiation of platelet responsiveness to nitric oxide by angiotensin-(1-7) is mediated by its specific receptor and is associated with the suppression of superoxide release. Accepted for presentation at ESC Congress, 2007

Scott R Willoughby, Sharmalar Rajendran, Elizabeth A Kelly, Tamila Heresztyn, Sue Leslie, Yuliy Y Chirkov and John D Horowitz.. Ramipril potentiates platelets nitric oxide responsiveness in patients with high cardiovascular risk. Accepted for presentation at ESC Congress, 2007

SR Willoughby, S Rajendran, EA Kelly, S Leslie, T Heresztyn, M Dronavalli, YY Chirkov, JD Horowitz. Hypertension is associated with impairment of platelet responsiveness to nitric oxide in a HOPE-type patient cohort. Accepted for presentation in Cardiac Society, CSANZ 2005

YY Chirkov, S Rajendran, JD Horowitz. Angiotensin-(1-7) improves platelet responsiveness to anti-aggregatory effects of nitric oxide. European Heart Journal, ESC Congress 2004

YY Chirkov, S Rajendran, JD Horowitz. Angiotensin-(1-7) potentiates the anti-aggregatory effects of nitric oxide. Accepted for presentation in AHA scientific sessions 2004.

Awards/grants relating to this thesis

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